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Evolution of the Chordate Muscle Actin Gene

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Summary. The ascidians *Styela plicata, S. clava,* and *Mogula citrina* are urochordates. The larvae of urochordates are considered to morphologically resemble the ancestral vertebrate. We asked whether larval and adult ascidian muscle actin sequences are nonmusclelike as in lower invertebrates, musclelike as in vertebrates, or possess characteristics of both. Nonmuscle and muscle actin cDNA clones from *S. plicata* were sequenced. Based on 27 diagnostic amino acids, which distinguish vertebrate muscle actin from other actins, we found that the deduced protein sequences of ascidian muscle actins exhibit similarities to both invertebrate and vertebrate muscle actins. A comparison to muscle actins from different vertebrate and invertebrate phylogenetic groups suggested that the urochordate muscle actins represent a transition from a nonmusclelike sequence to a vertebrate musclelike sequence. The ascidian adult muscle actin is more similar to skeletal actin and the larval muscle actin is more similar to cardiac actin, which indicates that the divergence of the skeletal and cardiac isoforms occurred before the emergence of urochordates. The muscle actin gene may be a powerful probe for investigating the chordate lineage.

Key words: Muscle actin gene — Urochordate — Ascidian — Chordate — Molecular evolution — Sequence comparison

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Actin is a highly conserved protein that forms the thin filaments in muscle fiber and the microfilaments found in all eukaryotic cells. Actins are thus categorized based on their functional roles as muscle actin or nonmuscle actin (also called cytoplasmic or cytoskeletal actin). Actin genes are often members of gene families which are differentially expressed in a tissue-specific manner during development (Davidson 1986). Mammals have four muscle actin isoforms (the sarcomeric skeletal and cardiac isoforms and the smooth enteric and vascular isoforms) and at least two nonmuscle isoforms (the β and γ cytoskeletal isoforms), although other nonmuscle actin types exist in amphibians and birds (Vandekerckhove and Weber 1978; Vandekerckhove et al. 1981; Bergsma et al. 1985). Based on comparisons among mammalian and avian muscle and nonmuscle actin sequences, 27 of 375 amino acid positions differentiate muscle and nonmuscle actins in vertebrates (Vandekerckhove and Weber 1984) and are referred to as diagnostic amino acids. However, the actins of fungi, protists, and both the muscle and nonmuscle actins from nonchordate invertebrate deuterstomes are more similar in sequence to vertebrate nonmuscle actins. The muscle actins of arthropods evolved independently and are distinct from vertebrate muscle actins (Mounier et al. 1992).

Because the diagnostic amino acids of muscle actin from nonchordate invertebrate deuterostomes are nonmusclelike (for example, the sea urchin *Centrostephanus robertsi*), and the diagnostic amino ac-

ids of muscle actin from a primitive vertebrate is musclelike (the lamprey *Lampetra fluviatilis*) (Vandekerckhove and Weber 1984), the evolution of vertebratelike muscle actin from a nonmusclelike progenitor probably began in the ancestral stock that gave rise to vertebrates. Many current theories maintain that a marine animal similar to a urochordate larva gave rise to vertebrates via pedogenesis (Bone 1981) because the larvae express all of the chordate features, and early embryos have similar fate maps to cephalochordates and many vertebrates (Garstang 1928; Berrill 1951).

The urochordates *Styela plicata, S. clava* and *Mogula citrina* are members of the phylum Chordata, subphylum Urochordata, and class Ascidiacea. The adults are sessile marine organisms with an elaborate pharynx for filter feeding but bear little resemblance to chordates. However, the motile larval state exhibits features characteristic of chordates, including gill slits, dorsal hollow nerve cord, notochord, and postanal tail. The larva is referred to as a tadpole due to its superficial resemblance to the amphibian tadpole. Following a short free-swimming period, the ascidian tadpole attaches to a substratum and undergoes metamorphosis into an adult. The larva has a muscular tail, and the adult has striated muscle underlying the tunic and siphons (Cavey 1983; Crowther and Whittaker 1984).

If the urochordate larva is representative of a vertebrate ancestor, then urochordate muscle actin may exhibit the transition from a nonmuscle to a musclelike actin sequence. We tested this hypothesis by analyzing the sequence of adult and larval muscle actin genes from ascidians.

Materials and Methods

Biological Materials. S. plicata were obtained from Marinus, Inc. (Long Beach, CA). Animals were maintained at 12° C in aquaria containing artificial seawater. The plasmid pBluescript II (Stratagene; San Diego, CA) was used for subcloning and DNA sequencing.

Isolation and Identification of the Actin Clones. The *S. clava* larval muscle actin clone ScTb1 was selected from a tailbud-stage complementary DNA (cDNA) library using the *S. plicata* muscle actin probe SpMA (Tomlinson et al. 1987a) as a probe. The nucleotide and deduced amino acid sequences are published (Beach and Jeffery 1992). The *S. plicata* muscle and nonmuscle actin clones were isolated from an adult muscle cDNA library prepared as described in Tomlinson et al. (1987a). The cDNA was synthesized from poly $(A)^+$ RNA isolated from the mantle muscle of the body wall and ligated to pBR322 via dG-dC tailing (Maniatis et al. 1982). Actin clones were selected using the *Drosophila melanogaster* actin clone DmA2 (Fyrberg et al. 1983). Other *S. plicata* primary muscle actin clones were obtained from M.E. White (University of Texas at Austin). These clones were also selected from an adult mantle cDNA library using SpMA as a probe. Double-stranded cDNA was ligated to λgt11 phage via *Eco*R1 linkers and packaged *in vitro* with Packagene extracts according to the manufacturer's instructions (Promega, Inc.; Madison, WI). The primary clones were purified by an additional round of duplicate screening (Sambrook et al. 1989). Sequences were determined by the dideoxy chain termination method (Sanger 1977) using the Sequenase DNA Sequencing Kit (USB; Cleveland, OH) and (^{35}S)-dATP (Dupont; Boston, MA) following the accompanying protocol. The *Styela* actin nucleotide sequences were deposited in EMBL, accession numbers X61040, X61041, and X61042. The *S. plicata* muscle actin sequence is referred to as SpMA1 and the nonmuscle actin sequence as SpCA8. The sequence of the *M. citrina* muscle actin cDNA clones is to be published elsewhere (M.E. White and W.R. Jeffery, personal communication).

A synthetic 17-mer oligonucleotide was used as a primer for sequencing part of the *S. plicata* muscle actin clone. It was synthesized on DNA synthesizer model 8600 (Biosearch Incorporated; San Rafael, CA) at the University of Houston and had the following sequence: 5'GCTTCAGTGAGGAGGAC3'.

Computer Analysis. Input of muscle actin sequences and database searching were performed using Genetics Computer Group Sequence Analysis Software Package (Devereux et al. 1984) run on a VAX computer (Digital Equipment Corporation) using the VMS operating system. Input was performed manually using SeqEd program. GenBank and EMBL database searching were performed using Strings and Fetch programs. DNA and protein sequence data were manually aligned and the database was maintained on a sequence editing program called Seqedt. Phylogenetic analysis to generate phenograms via maximum parsimony was implemented with the PAUP 3.0r computer package (Swofford 1991). Six heuristic searches using the general option were utilized, and strict consensus trees were used for the figures.

Results and Discussion

Ascidian Actin Sequences

Muscle and nonmuscle actin clones were selected from an *S. plicata* cDNA library prepared from polyadenylated RNA isolated from adult mantle muscle. The nucleotide sequence of the *S. plicata* adult muscle actin clone (Fig. 1) was found to be identical to a partial muscle actin cDNA clone published earlier called SpMA (Tomlinson et al. 1987a). The clone described in this paper is referred to as SpMA1. SpMA1 was determined to be a muscle actin clone by several criteria; the partial deduced amino acid sequence was more musclelike than nonmusclelike (see Table 1), the protein product from hybrid selection experiments migrated to the acidic actin isoform position on two-dimensional gels, and the temporal and spatial expression of SpMA1 RNA showed it to be muscle specific (Tomlinson et al. 1987a,b). Another actin gene from *S. plicata* named SpCA8 was cloned and sequenced (Fig. 1). Based on the deduced amino acid sequence, the diagnostic amino acids clearly show SpCA8 to be nonmusclelike. (See Table 1.) The SpMA1 and SpCA8 clones show that ascidians pos-

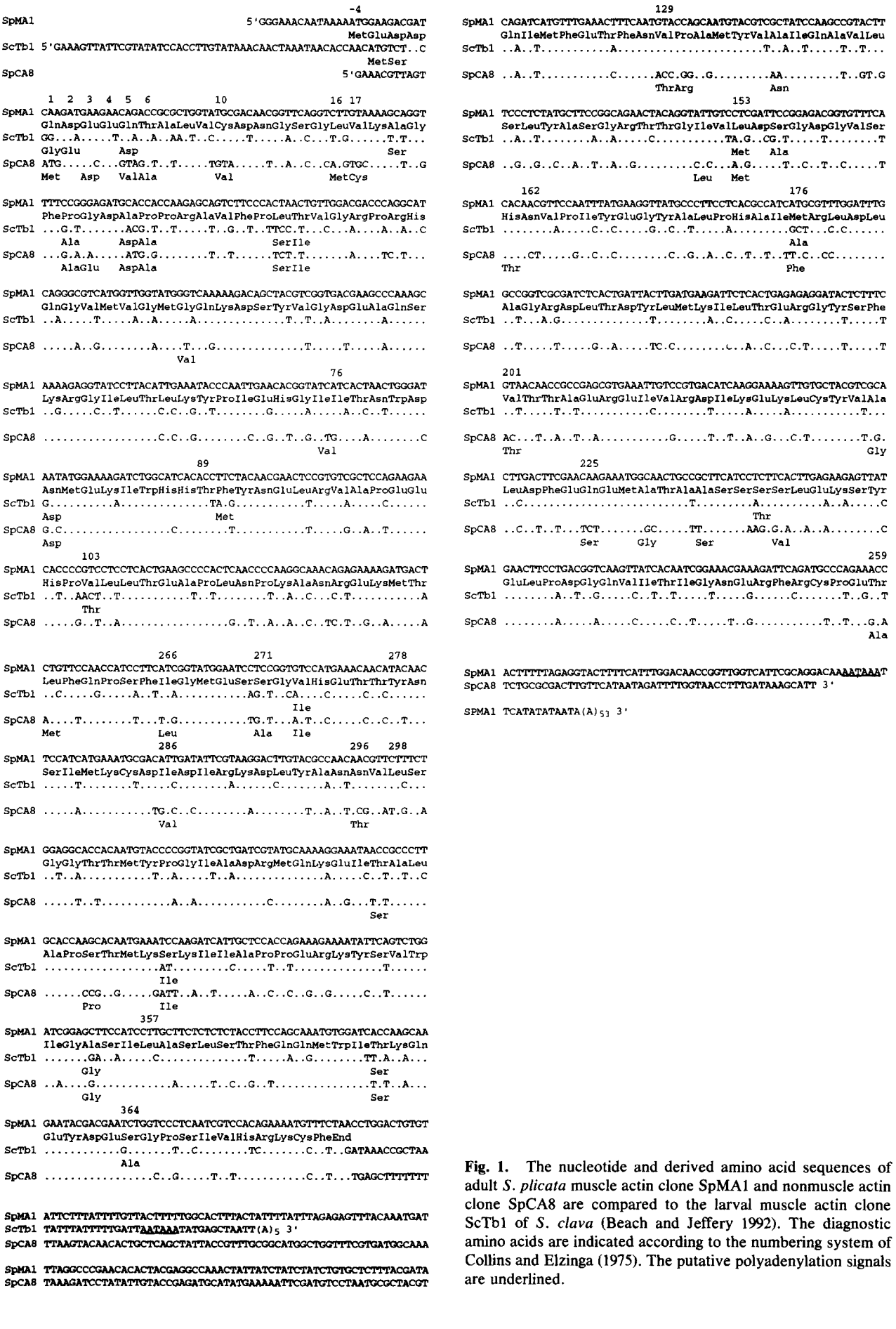

Fig. 1. The nucleotide and derived amino acid sequences of adult *S. plicata* muscle actin clone SpMA1 and nonmuscle actin clone SpCA8 are compared to the larval muscle actin clone ScTb1 of *S. clava* (Beach and Jeffery 1992). The diagnostic amino acids are indicated according to the numbering system of Collins and Elzinga (1975). The putative polyadenylation signals are underlined.

Table 1. A comparison of the diagnostic amino acids from muscle and nonmuscle actins of various animal groups and species[a]

SOURCE	Actin Type	Diagnostic Amino Acid (Position Number) 1	2	3	4	5	6	10	16	17	76	89	103	129	153	162	176	201	225	259	266	271	278	286	296	298	357	364	Actin Group	Reference
Vertebrate Chordates																														
Mammals	Skeletal	D	E	D	E	T	T	C	L	V	I	T	T	V	L	N	M	V	N	T	I	A	Y	I	N	M	T	A		6
&	Cardiac	D	D	E	E	T	T	C	L	V	I	T	T	V	L	N	M	V	N	T	I	A	Y	I	N	L	S	A		6
Birds	Vascular	E	E	E	D	S	T	C	L	C	I	S	T	V	L	N	M	V	N	T	I	A	Y	I	N	L	S	A		6
	Enteric	–	E	E	E	T	T	C	L	C	I	S	T	V	L	N	M	V	N	T	I	A	Y	I	N	L	S	A	A	6
	Skeletal (α 3)	D	D	D	E	T	T	C	L	V	I	T	T	V	L	N	Q[N]	V	N	T	I	A	Y	I	N	L	T	A		7
[1]Amphibians	Cardiac (α 1)	D	D	E	E	T	T	C	L	V	I	T	T	V	L	N	Q[N]	V	N	T	I	A	Y	I	N	L	S	A		7
Lamprey	Muscle	D	D	E	E	T	T	C	L	C	I	T	T	V	L	N	M	V	N	T	I	A	Y	I	N	L	T	A		8
Invertebrate Chordates (Urochordates)																														
Styela clava	Larval Muscle	G[M]	E	E	D	Q[M]	T	C	L	V	I	T	T	V	M	N	A[C]	V	Q	T	I	A	Y	I	N	L	S	A		
Styela plicata	Adult Muscle	Q[N]	D	E	E	Q[M]	T	C	L	V	I	T	V	V	L	N	M	V	Q	T	I	S[C]	Y	I	N	L	T	S	B	The Authors
[2]Molgula citrina	Adult Muscle	–	–	E	E	Q[M]	T	C	L	V	I	T	V	V	L	N	M	V	Q	T	I	S[C]	Y	I	N	L	T	S		
Invertebrates																														
Sea Urchin	Muscle	–	D	D	E	V[C]	A	V	M	C	V	T	V	A[M]	L	T	L	T	Q	T	I	A	Y	I	T	L	S	S		8
[3]Drosophila melanogaster	Muscle (79B)	C[N]	D	E	E	A[C]	S[M]	V	M	C	I	T	V	S[C]	L	T	L	T	Q	A	L	C	Y	V	N	L	S	S	C	9
Scallop	Muscle	–	D	D	E	V[C]	A	V	M	C	V	T	V	A[M]	L	T	L	T	N	S[M]	L	A	Y	V	T	L	S	S		8
[4]Nonmuscle																														
Mammal	β-isoform	–	D	D	D	I	A	V	M	C	V	T	V	T	M	T	L	T	Q	A	L	C	F	V	T	L	S	S		6
	[5]γ-isoform		E	E	E			I																						6
Amphibian	β-isoform	A[N]	D	D	D	I	A	I	M	C	V	T	V	T	M	T	L	T	Q	A	L	C	F	V	T	L	S	S		10
Styela plicata		M[N]	D	D	E	V[C]	A	V	M	C	V	T	V	T	M	T	F[N]	T	S[N]	A	L	A	Y	V	T	L	S	S	D	The Authors
Sea urchin	Cyl	C[N]	D	D	D	V[C]	A	I	M	V	V	T	V	S[C]	F[N]	T	L	T	Q	A	L	A	Y	V	T	L	S	S		11
Drosophila melanogaster	5c	C[N]	D	E	E	V[C]	A	V	M	C	V	T	V	T	L	T	L	T	Q	A	L	C	Y	V	T	L	S	S		12

[a] The position numbers of the diagnostic amino acids are based on the numbering system of Collins and Elzinga (1975). The thin boxes represent amino acids found in both muscle and nonmuscle actins; the bold boxes represent replaced amino acids where the superscript M is a musclelike replacement, C is a nonmusclelike replacement, and N is neither a musclelike or nonmusclelike replacement; the circled letters represent nonmusclelike amino acids; and the bold letters represent musclelike amino acids. Abbreviations: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparginine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan, Y, tyrosine. Footnote numbers: [1]Only one of the two *Xenopus laevis* skeletal muscle actins is shown. [2]A partial muscle actin sequence from adult *Molgula citrina* is identical to *S. plicata* at diagnostic amino acid positions 3 through 364. Diagnostic amino acid 1 is unknown and diagnostic amino acid 2 is a glutamic acid instead of an aspartic acid (M.E. White and W.R. Jeffery, unpublished data). [3]Only one of the four *D. melanogaster* muscle actins is shown.[4]Only representative nonmuscle actins are shown. [5]The β and γ nonmuscle actins are identical except where indicated. [6]Vandekerckhove and Weber (1979). [7]Stutz and Spohr (1986). [8]Vandekerckhove and Weber (1984). [9]Sanchez et al. (1983). [10]Cross et al. (1988). [11]Cooper and Crain (1982). [12]EMBL accession numbers K00667, K00668, K00669.

sess both musclelike and nonmusclelike actin sequences.

In Fig. 1, the deduced amino acid sequences of SpMA1 and SpCA8 are compared to the *S. clava* larval muscle actin cDNA clone ScTb1 (Beach and Jeffery 1992). The numbers above the sequences show the positions of the diagnostic amino acids, which distinguish musclelike and nonmusclelike actins (Vandekerckhove and Weber 1978). There are 206 nucleotide differences (18.3%) in the coding region of the two *Styela* muscle actins SpMA1 and ScTb1. There are 21 of 376 (5.6%) amino acid replacements, including nine of the 27 diagnostic amino acids. (See Table 1.) Table 1 also includes an actin sequence from the ascidian *M. citrina* (M.E. White and W.R. Jeffery, personal communication). It is identical to the *S. plicata* muscle actin sequence at diagnostic positions 3 through 364.

Ascidian Muscle Actin Has Characteristics of Muscle and Nonmuscle Actins

Based on mammalian and avian actin sequences, Vandekerckhove and Weber (1984) found that the muscle actins of vertebrates are highly conserved and differ from invertebrate muscle actins at specific diagnostic amino acids. The diagnostic amino acids showed that the muscle actins of invertebrates are more similar in sequence to vertebrate nonmuscle actin than muscle actin. Table 1 shows a comparison of the diagnostic amino acids for representative actins from different protostome and deuterostome sources in roughly descending order from the muscle actin standards (mammalian and avian muscle actins) to the nonmuscle actin standards (mammalian β and γ nonmuscle actins). The differences among the vertebrate muscle actins are slight. Table 1 shows that the same muscle actin isoforms from different vertebrate classes are more similar than are different muscle actin isoforms within a species or class. For example, the number of diagnostic amino acid changes between mammalian skeletal and enteric smooth actins is seven, whereas only three changes are found between mammalian skeletal actin and a skeletal muscle actin isoform identified in *Xenopus laevis* (class Amphibia).

The ascidian muscle actins are primarily musclelike, yet display distinct nonmusclelike features. Diagnostic positions 103, 225, 271, and 364 from *S. plicata* and *M. citrina* adult muscle and positions 153, 176, and 225 from *S. clava* larval muscle are nonmusclelike. There are few examples of a vertebrate muscle actin containing nonmusclelike diagnostic amino acids. (Most of the examples occur at positions 2, 3, and 4 and probably should not be considered diagnostic; see Table 1.) There are distinct differences in actin sequences depending on whether they are derived from vertebrates, chordate invertebrates, nonchordate invertebrates, or nonmuscle tissue. These distinctions reaffirm the notion that the urochordate muscle actin is an evolutionary intermediate between invertebrate and vertebrate muscle actin.

Evolution of Skeletal and Cardiac Actin Isoforms

Although the urochordate muscle actin has nonmuscle characteristics, the protein is decidedly more similar to sarcomeric muscle (the skeletal and cardiac isoforms) than smooth muscle (the vascular and enteric isoforms) as illustrated by positions 17 and 89, the two positions that differentiate sarcomeric and smooth muscle (Table 1). This suggests that the prototype vertebrate muscle actin was sarcomeric in nature, which is consistent with the single known muscle actin isoform identified in lamprey (Vandekerckhove and Weber 1984).

The two *Styela* muscle actins differ at nine of the 27 diagnostic positions. These changes may simply reflect species differences, but this is highly unlikely for such a conserved protein in two closely related species. The high dissimilarity between the *Styela* larval and adult muscle actins has an interesting implication regarding the divergence of sarcomeric actins. The *S. clava* tadpole muscle actin more closely resembles cardiac actin as shown by amino acids leucine and serine at positions 298 and 357 (the two diagnostic positions that distinguish skeletal and cardiac actins), while the muscle actin of adult *S. plicata* shows a greater similarity to skeletal actin as shown by the threonine at position 357.

It was previously proposed that the sarcomeric actins diverged during the emergence of amphibians (Vandekerckhove and Weber 1984), and although we have not yet identified more than one muscle isoform per ascidian species, the data indicate the skeletal/cardiac divergence took place prior to the appearance of urochordates. We predict the presence of both sarcomeric isoforms in lower vertebrates, perhaps expressed at different times during the life cycle.

The data also suggest that the evolution of vertebrate muscle actin was probably well underway in the vertebrate ancestor. For example, there are 18 differences between the diagnostic amino acids of sea urchin muscle and mammalian skeletal actins, but only nine differences between *Styela* muscle and mammalian skeletal actins. Therefore, evolution of muscle actin from a nonmuscle actin sequence probably began early in the protochordate lineage.

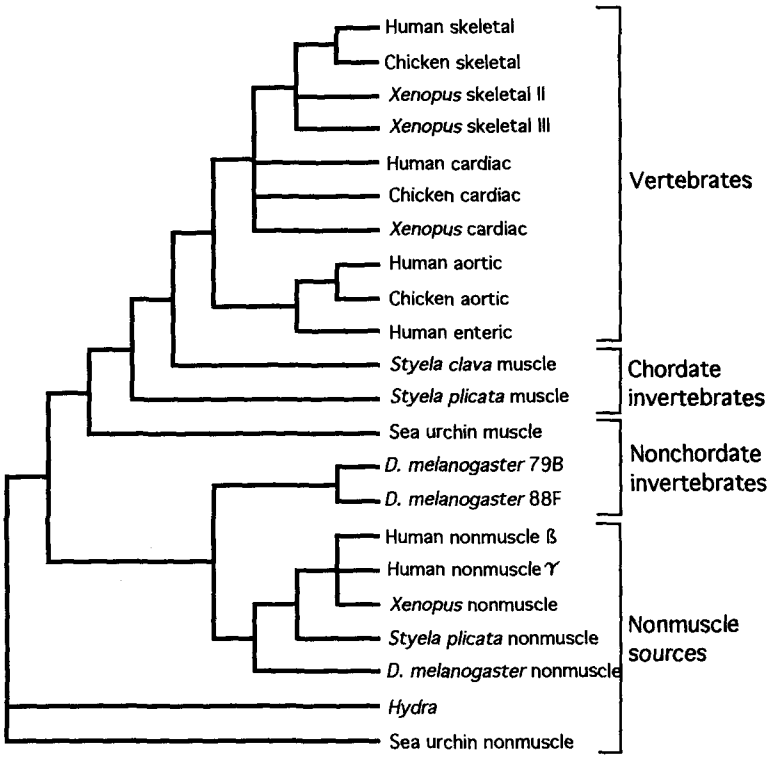

Fig. 2. Phylogenetic tree of the aligned derived amino acids from the codogenic regions of 22 actin genes. Phylogenetic analysis to generate phenograms via the parsimony method was implemented with the PAUP Computer Package (PAUP 3.0r) (Swofford 1991). The actin sequence from *Hydra attenuata* (Fisher and Bode 1989) was used as an outgroup to root the trees. Note that the sea urchin nonmuscle actin is divergent enough to cluster with the *Hydra* outgroup. Human skeletal muscle (EMBL accession #J00068); human cardiac muscle (EMBL accession #J00070); human aortic muscle (EMBL accession #X13839); human enteric muscle (EMBL accession #16940); chicken skeletal muscle (EMBL accession #s J000805, K02172, K02257); chicken cardiac muscle (EMBL accession #M13756); *Xenopus* skeletal muscle II (EMBL accession #s X05392, Y00072); *Xenopus* skeletal muscle III (EMBL accession #X12525); *Xenopus* cardiac muscle (EMBL accession #X04669); *Styela clava* muscle; *Styela plicata* muscle; sea urchin muscle (EMBL accession #X05739); *D. melanogaster* muscle, locus 79B (EMBL accession #s M18829, J01064); *D. melanogaster* muscle, locus 88F (EMBL accession #s M18830, J01065); human nonmuscle β (EMBL accession #s X00351, J00074, M10278); human nonmuscle γ (accession #M19283); *Xenopus* nonmuscle (EMBL accession #X07510), *Styela plicata* nonmuscle, sea urchin nonmuscle (EMBL accession #M35324); *D. melanogaster* nonmuscle (EMBL accession #s K00667, K00668, K00669).

Phylogenetic trees were generated by heuristic analysis using the aligned protein sequences from the codogenic regions of 22 actin genes (Fig. 2). The sequence of *Hydra attenuata* was used as an outgroup to root the trees. The phylogenetic tree shows that the different actins group according to whether they came from vertebrate muscle (1), protochordate muscle (2), or invertebrate muscle and nonmuscle sources (3 and 4). The tree shows that *Styela* actins fall between the vertebrate and nonchordate invertebrate actins.

The muscle actin gene appears to be an excellent probe for delineating the vertebrate lineage. Figure 3 illustrates our concept of the evolution of the chordate actin genes. The evolution of the chordate muscle actin gene may have begun in an early echinoderm-like ancestor. Table 1 shows that at least two different actin genes evolved in echinoderms, each encoding actins with muscle or nonmuscle functions. Sea urchin muscle actin has six unambiguous musclelike diagnostic amino acids, but sea urchin nonmuscle actin has only three musclelike diagnostic amino acids. These data suggest that two different actin genes from an early echinoderm gave rise to the respective muscle and nonmuscle actin genes in chordates.

The two early actin gene types probably had a common ancestor. This assertion is based on common intron placement (Dibb and Newman 1989). For example, the placement of the four introns in the coding region of the single muscle actin gene in *Strongylocentrotus purpuratus* (Crain et al. 1987) is identical to four of the seven intron positions in human muscle actin, to three of the four in human nonmuscle actin, and to two of the four in *S. purpuratus* nonmuscle genes (Dibb and Newman 1989). The intron placements in protostomes and lower taxonomic groups, with few exceptions, are different. The early protochordate muscle actin gene then evolved rapidly toward a sarcomericlike actin, duplicated, and diverged further to give rise to skeletal and cardiac actins. No smooth muscle actins have been found in protochordates nor agnathans; thus the divergence of the smooth muscle actin isoforms from a cardiac ancestor is probably as previously described (Vandekerckhove and Weber 1984). Based on the diagnostic amino acids, the nonmuscle actins have remained relatively unchanged from echinoderms to mammals.

The skeletallike actin of *Styela plicata* is expressed in the adult muscle underlying the mantle, but it is also expressed in the tail muscles during later embryonic stages (Tomlinson et al. 1987a,b). The cardiaclike actin of *S. clava* is expressed in the

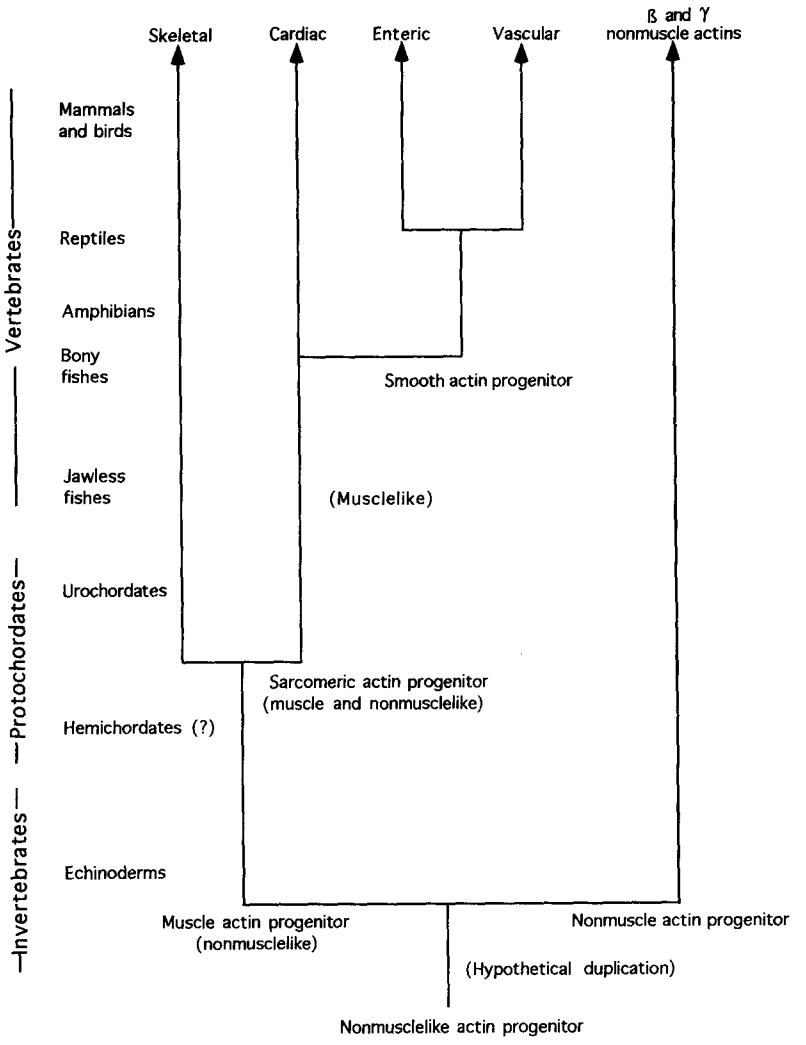

Fig. 3. A depiction of the earliest taxonomic groups in which the actin genes are thought to have duplicated and diverged. Each branch point represents a duplication event. A nonmuscle-like progenitor, perhaps similar to the muscle actin in echinoderms, evolved rapidly and gave rise to a sarcomeric form of actin, which duplicated and diverged slightly to give the skeletal and cardiac progenitors evident in urochordates. The question mark by hemichordates indicates the lack of sequence data as to whether the duplication occurred before or after their appearance. We have no further evidence as to when the smooth muscle progenitors diverged, and they are placed according to Vandekerckhove and Weber (1984). The nonmuscle actins have changed little from the nonmuscle forms seen in echinoderms. Internode distances do not imply absolute evolutionary distance.

mesenchyme and muscle cell lineages during embryogenesis but is also expressed in adult muscle (Beach and Jeffery 1992). The coexpression of cardiac and skeletal actins in the same adult tissues is analogous to what is found in vertebrates (Gunning et al. 1983; Mayer et al. 1984; Paterson and Eldridge 1984; Stutz and Spohr 1986; Vandekerckhove et al. 1986; Alonso 1987).

Also, as in vertebrates, cardiac actin is the major sarcomeric isoform expressed in embryonic striated muscle (Baines et al. 1984; Paterson and Eldridge 1984; Stutz and Spohr 1986; Hayward and Schwartz 1986; Vandekerckhove et al. 1986; Mohun et al. 1988; Sassoon et al. 1988). The larval tail muscle cells of *Diplosoma macdonaldi* and *Styela* are striated (Cavey 1983; Crowther and Whittaker 1983; W.R. Jeffery, unpublished observations), and the expression of cardiac actin as the primary sarcomeric form in *S. clava* embryonic muscle cells is consistent with observations made in vertebrates.

There are at least four and possibly as many as seven muscle actin genes in *S. clava* which encode identical cardiaclike actins (Beach and Jeffery 1992). It has been proposed that some members of the sea urchin actin multigene family originated by gene duplication, and the similar coding regions were maintained by gene conversion (Crain et al. 1987; Durica et al. 1988). It is likely that the *S. clava* muscle actin genes arose in the same way. The *S. clava* muscle actin genes are independently regulated during embryogenesis, each with a different temporal and spatial mode of expression (Beach and Jeffery 1992), similar to the type of transcriptional regulation found in *Drosophila melanogaster* and sea urchin embryos (Fyrberg et al. 1983; Shott et al. 1984; Cox et al. 1986).

In conclusion, ascidian muscle actins are similar to vertebrate muscle actins in that they have relatively high identity, share common introns, and are coexpressed in adult tissues, and cardiac actin is the major isoform expressed in embryos. However, the number of actin genes and their transcriptional regulation are reminiscent of invertebrates in that multiple genes encoding the same or similar isoforms are expressed during development. The muscle actins of ascidians therefore not only show the tran-

sition from invertebrates to vertebrates at the structural level but also at the level of regulation.

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